(12) UK Patent Application (19) GB (11) 2 136 130 A

(43) Application published 12 Sep 1984

(21) Application No 8402600

(22) Date of filing 1 Feb 1984

(30) Priority data

(31) 83470259 (32) 28 Feb 1983 (33) US

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(51) INT CL³
G01N 27/22 C12Q 1/00 G01N 31/00 33/50

(52) Domestic classification
G1N 1A3B 1D5 1F 1M1 7F 7K AEN
G1B BU
U1S 1296 1333 1335 2159 G1N

(56) Documents cited
GB A 2008767
GB 1586117
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GB 1418388

(58) Field of search
G1N

(54) Chemical Assay Systems

(57) Apparatus and methods for chemical assay employ a thin-film capacitor 12, 14 overcoated at least in part by a thin layer 26 of a material chosen to be reactive with the species to be detected or quantitated. To perform an assay, the capactive transducer is contacted by a fluid sample to be assayed. Reaction of the sample with the reactive layer overcoating the capacitor results in a change in the capacitance of the capacitor. A non-reactive insulating layer 24 may be present & electrodes 12, 14 may be interdigitated. The layer 26, or the material tested for, may be biologically active, or inorganic.

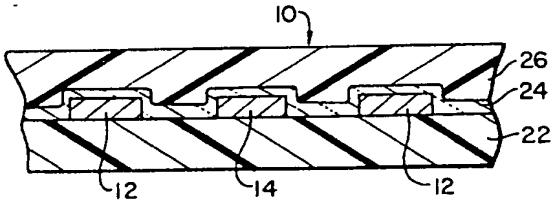

FIG. 2

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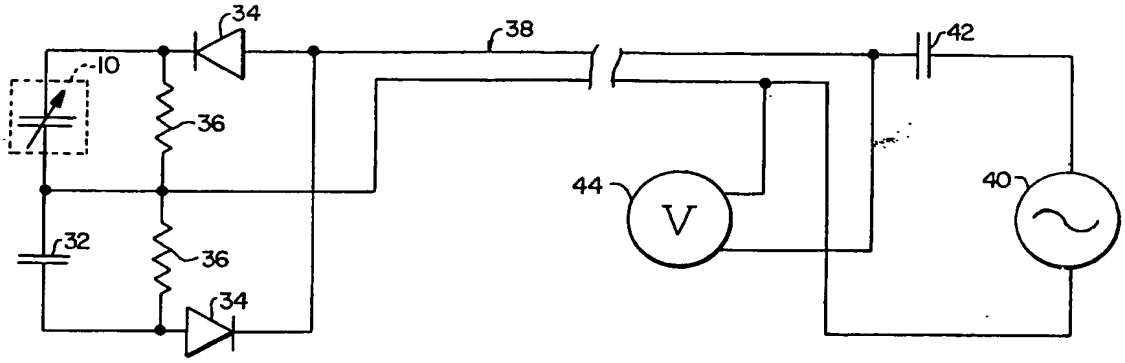

FIG. 3

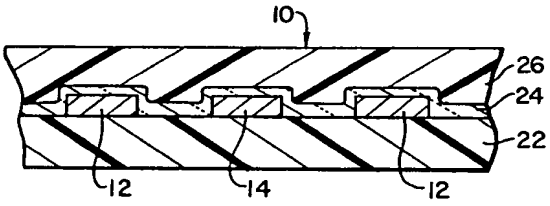

FIG. 2

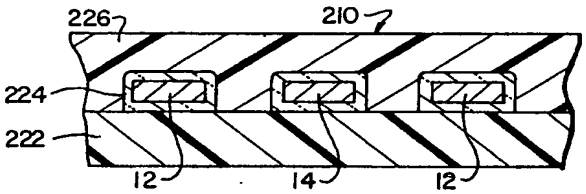

FIG. 5

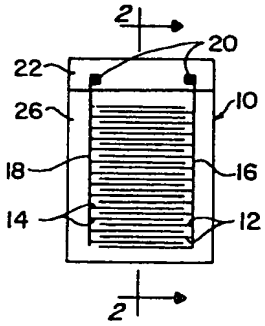

FIG. 1

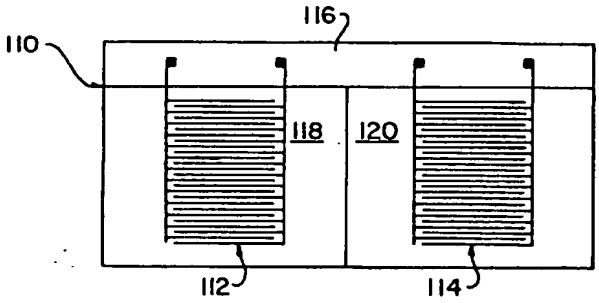

FIG. 4

SPECIFICATION

Chemical Assay Systems and Methods

Background of the Invention

This invention relates to methods and apparatus for the assay of chemicals and chemical systems, and more particularly to electrical methods and apparatus wherein a specific chemical reaction is detected and quantitated as a result of a change in the dielectric properties of the reacting system.

It is to be understood that for the purposes of the present invention, chemical and chemical system assay is meant to include not only the qualitative and quantitative assays common to physical and organic chemistry, but also bioassays wherein such biochemical reactions as immunological, enzyme, or metabolic reactions are detected and quantitated.

It is well known that the dielectric properties of a sample may be useful in the chemical assay of the sample. For example, the concentration of the constituents of the solution of two materials having different relative dielectric constants may be readily determined by measuring the dielectric constant of the solution. Typically, the dielectric constant of the sample is measured by capacitive means, the sample providing all or part of the dielectric material separating the plates of a capacitor. From the geometry of the capacitor and the measured capacitance, the dielectric constant of the sample may be deduced. This value may then be compared with a calibration curve or table of values of the dielectric constant for varying known concentrations of the materials of interest. Typically, such values may be determined by directly substituting known concentrations of the materials in the capacitor. Beyond detecting and quantitating a species of interest (e.g., an element, compound, ion, or the like), the method may be extended to observing reaction rates of interacting species.

Although the samples may be introduced between the plates of a parallel plate capacitor, a convenient method juxtaposes the sample and a capacitor having both electrodes in a common plane, as disclosed, for instance, in U.S. Patents 2,219,497 and 3,515,987. Measurements are generally made by reasonance methods, by an impedance bridge, or the like. The resulting output signal may be used to drive a meter, a digital display, or similar apparatus. In other applications, the signal may be observed by one or more pre-set level detectors and used for alarm or control purposes. It will be appreciated that such equipment provides a particularly convenient, easy to use, system, requiring little skill on the part of the user.

While such techniques have found application in process control (e.g., humidity monitors in paper manufacturing, monitoring electrolytic solutions in batteries or in electroplating operations, and the like) or similar controlled situations, the technique as so far practiced lacks universality. Capacitive measurements alone are generally insufficient to unambiguously identify substances in a multi-part or an unknown mixture, or to quantitate their concentrations or reactivities.

Further, for many assays, the material to be detected or quantitated may be present in very small concentrations in the presence of other materials (frequently of much larger and also variable concentrations) that also affect the dielectric constant of the sample. The presence of such interefering substances clearly limits the usefulness of an otherwise simple measurement technique, inasmuch as both the detection threshold and the quantitation precision may be unfavorably impacted by the relative concentrations of the substance to be assayed and the interfering substances. Thus, large concentrations of interefering substances in increasing the total signal (background plus signal of interest) may also increase the noise level in the signal, thereby decreasing the accuracy of detection or quantitation of the substance of interest even if the interfering substance has a known and constant concentration. The situation is clearly exacerbated by an unknown variable concentration of an interfering substance.

Objects of the Invention

Accordingly, it is an object of the present invention to provide methods and apparatus for simple and unambiguous identification and quantitation of specific substances through capacitive measurement of the dielectric properties of a medium.

It is also an object of the invention to provide methods and apparatus for such assays that are relatively immune to the effects of large or varying concentrations of interfering substances.

Brief Description of the Invention

These and other objects are met in the present invention of a thin-film capacitor overcoated at least in part by a thin layer of a material chosen to be reactive with the species to be detected or measured. To perform an assay, the capacitive transducer is inserted into a fluid sample to be qualitatively or quantitatively assayed or in which the reaction kinetics are to be observed.

While any reactant or catalyst may be used to form the overcoating layer, biochemical reactants generally exhibit the highest specificity. Thus, a preferred embodiment, intended for enzyme assays, is in the form of a thin film capacitor overcoated with a thin layer of the biological substrate of the enzyme of interest. The change of the biological substrate brought about by enzymatic catalysis produces a change in capacitance which may be used to indicate the presence of and to quantitate the concentration of the enzyme in the sample.

In another embodiment, intended to detect and quantitate specific antigens, haptens, antibodies, or antibody fragments, a thin layer of a material immunologically reactive with the species to be quantitated is immobilised to the capacitive

structure. In this embodiment, the formation of antibody-antigen complex immobilized to the capacitor alters the capacitance, allowing detection and quantitation of antigen or antibody.

Other embodiments include the use of a microbiological growth medium as the reactive layer (to detect and quantitate microbiological metabolism) and the use of a simple chemically reactive layer (to detect a class of chemicals or chemical reactions).

Reaction of the sample with the reactive layer overcoating the capacitor results in a change in the capacitance of the capacitor. The rate of accumulation of reaction products, and therefore the rate of change in capacitance, depends, *inter alia,* on the concentration of the reacting species. Interefering background substances in the sample, while contributing to the total capacitance of the system, do not enter into the reaction. Consequently, by observing the rate of change of the capacitance of the system, the concentration of the species of interest may be deduced without knowledge of the concentration of the interefering substances. The apparatus may also be used in a non-ballistic mode, by observing the capacitance at the end-point of the reaction or at a set time after exposure, provided the effect of interfering substances is small or otherwise known.

It will be appreciated that the apparatus so far described has the convenience of a simple electrical transducer that, depending on the specificity of the reaction, may be made highly specific for a desired material and relatively insensitive to interfering substances likely to be present in a sample.

Other objects of the invention will in part be obvious and will in part appear hereinafter. The invention accordingly comprises the apparatus possessing the construction, combination of elements, and arrangement of parts and the several steps and the relation of one or more of such steps with respect to each of the others which are exemplified in the following detailed disclosure and the scope of the application of which will be indicated in the claims.

Brief Description of the Drawings

For a fuller understanding of the nature and objects of the present invention, reference should be had to the following detailed description taken in connection with the accompanying drawings wherein:

Figure 1 is a front view of a transducer which is a preferred embodiment of the present invention;

Figure 2 is an enlarged fragmentary cross-sectional view of a portion of a transducer of Figure 1 taken along the line 2—2 of Figure 1;

Figure 3 is a schematic diagram of circuitry suitable for use with the transducer of Figures 1 and 2;

Figure 4 is a front view of an alternative embodiment of the transducer of the present invention; and

Figure 5 is a view, similar to that of Figure 2, of an alternative embodiment of the transducer of Figure 1.

In the various views, like index numbers refer to similar elements.

Detailed Description

Referring to Figures 1 and 2, there may be seen a transducer 10 which constitutes a preferred embodiment of the present invention. Transducer 10 is preferably in the form of an interdigitated capacitor formed of a plurality of parallel equally spaced-apart electrodes 12 interdigitated with a similar plurality of parallel electrodes 14. A pair of parallel spaced-apart busses 16 and 18, disposed normal to the electrodes, are electrically connected to opposite ends of electrodes 12 and 14 respectively. Busses 16 and 18 are each provided with an electrically connected contact pad 20 remoted from electrodes 12 and 14.

Electrodes 12 and 14, busses 16 and 18, and contact pads 20 are fabricated of an electrically conductive thin metalic film, such as chromium, aluminum, tungsten, titanium, tantalum, platinum, paladium, or the like. As is well known in the art, microcircuits of such thin films may be deposited upon a suitable mechanical support 22 by any of a number of processes, such as evaporation, sputtering, low pressure chemical vapor deposition (LPCVD), plasma deposition, or the like. Mechanical support 22 is preferably fabricated as a smooth, polished, flat, electrically insulating plate made, for instance, of sapphire, quartz, glass, or alumina. However, as will become apparent hereinafter, for certain applications mechanical support 22 may be foraminous, so as to permit circulation of a sample fluid through transducer 10. Then, too, mechanical support 22 may be a polymeric material, and electrodes 12 and 14, busses 16 and 18, and contact pads 20 may be printed thereon, as by silk screen techniques.

In a preferred embodiment electrodes 12 and 14, busses 16 and 18, and contact pads 20 are grown from tungsten on a sapphire support 22 from the reaction of tungsten hexafluoride and hydrogen at a temperature of 720°C in an LPCVD reactor. The metal is grown to a thickness of about 2000 Angstroms. The capacitor's electrically conductive structure is delineated by standard photoetching techniques, the tungsten first being coated with a photoresist which is then exposed with the desired pattern. After development, the photoresist is washed to remove the unpolymerized portions of the resist, and the uncovered tungsten is chemically dissolved by an appropriate etchant. After etching, the resist is removed with an appropriate solvent, leaving the tungsten electrode pattern on the sapphire substrate. Each electrode is on the order of 0.001 inch (0.025 mm) wide, disposed such that opposing electrodes are spaced apart by a similar distance (i.e., electrodes 12 or electrodes 14 are spaced apart from like electrodes by 0.003 inch [0.075 mm]). Satisfactory electrical performance has been achieved on a saphire

substrate with 80 tungsten electrodes (40 each of electrodes 12 and 14) and a spacing between busses 16 and 18 of 0.140 inch (3.57 mm), resulting in capacitors having capacitances (before additional treatment) on the order of 10 to 15 picofarads. It will be understood, however, that for varying applications, both the form and dimensions of the capacitor, and its capacitance, might be varied.

As may best be seen in Figure 2, transducer 10 is further overcoated with a dielectric layer 24. Dielectric layer 24 is chosen not only for its dielectric properties but also so as to be impervious to the fluids of interest. Dielectric layer 24 serves as a mechanical, electrical, and chemical protective barrier over electrodes 12 and 14 and busses 16 and 18 of the transducer. Dielectric layer 24 may be composed of any of a number of well known materials, such as silicon nitride, silicon dioxide, silicon oxynitride, aluminum oxide, or the like. As an example, silicon nitride can be grown by LPCVD through the reaction of silane (SiH_4) and ammonia (NH_3). Satisfactory transducers have been made with a silicon nitride dielectric layer on the order of 3500 Angstroms thick. Alternatively, dielectric layer 24 may be glass, deposited as by sputtering in an argon and oxygen atmosphere at low pressure (e.g., 10 to 20 microns). Glass may also be grown by chemical vapor deposition through the reaction of silane (SiH_4) and nitrous oxide (N_2O) or carbon dioxide (CO_2) grown in an LPCVD reactor. Those skilled in the art will appreciate that any number of other materials, including polymeric materials, may be employed to produce dielectric layer 24.

As will be described hereinafter in greater detail, transducer 10 is further provided with a reactive overcoating 26 covering, at least in part, electrodes 12 and 14. Reactive overcoating 26 is chosen on the basis of its reactivity with the specific material to be detected or quantitated, or because it enters into the specific reaction that is to be studied. Thus, reactive overcoating 26 is a reactant or a catalyst involved in a reaction with the material to be detected or quantitated. It will be understood that reactive overcoating 26 is also preferably selected so as to not be reactive with other substances likely to be present in the sample that may tend to mask the presence or spoil the quantitation of the species of interest.

While the present invention may be practiced with reactants or catalysts of greater or lesser specificity for the material or reaction of interest, it will be appreciated that the higher the specificity of reactive overcoating 26 becomes, the less likely is an erroneous detection or quantitation. Generally, the most specific reactants are biochemical in nature, and consequently, a preferred embodiment of the present invention employs a biochemically reactive overcoating 26. Thus, in a preferred embodiment, reactive overcoating 26 comprises a coating of an antibody or a reactive antibody fragment (Fab) for the assay of an antigen or hapten (including hormones, alkaloids, steroids, and the like). Alternatively, for the assay of antibody (or Fab), reactive overcoating 26 may be an antigen or hapten. Yet again, for enzyme assays, an appropriate biochemical substrate is used as the reactive overcoating.

Transducer 10 is employed with appropriate circuitry to permit the determination of changes in the capacitance of the transducer occuring as a result of reactions between reactive overcoating 26 and the sample. An example of a suitable bridge circuit for use with transducer 10 is illustrated in the schematic diagram of Figure 3. This particular circuit, which will be described here only to the extent necessary to make clear the operation of sensor 10, was originally described by D. R. Harrison, W. J. Kerwin, and G. L. Shaffer in *The Review of Scientific Instruments,* Vol. 41, No. 12, pp. 1783 ff, (December, 1970). Transducer 10 (schematically shown as a variable capacitor) and reference capacitor 32 are connected with diodes 34 and equal resistances 36 to form a diode-impedence bridge. Diodes 34 are attached in parallel and with opposite polarity to one side of twin lead 38. The same side of twin lead 38 is coupled to signal generator 40 through coupling capacitor 42. Voltmeter 44 is connected across the twin lead in parallel to signal generator 40 and coupling capacitor 42. The second line of the twin lead is center-tapped between the series-connected resistors 36 and the parallel series connected transducer 10 and reference capacitor 32 connected to diodes 34. When signal generator 40 is activated, a D.C. voltage appears across voltmeter 44 which is directly proportional to the difference in capacitance between transducer 10 and reference capacitor 32 and inversely proportional to the sum of the same two capacitances.

It will be understood, however, that one or more transducers 10 may be employed in combination with other circuitry in order that the dielectric properties of the medium adjacent or reacting with overcoating 26, or the reaction products of such reaction, may be measured.

In operation, reactive overcoating 26 of transducer 10 is placed in contact with a sample of fluid to be assayed. Reaction between reactive overcoating 26 and the fluid sample results in a change of the composition of reactive overcoating 26 and the sample adjacent the overcoating (or the sample alone if the reactive overcoating is a catalyst). As a result of these changes, the capacitance of the transducer may be observed to change, since, in the more general case, both the dimensions and the dielectric constant of reactive overcoating 26 and the adjacent fluid change. In the simplest case, the rates at which these changes occur are limited by diffusion, and, consequently, by the concentration of the reacting species.

EXAMPLES

The following examples are offered by way of illustration and not by way of limitation.

In each of the examples, unless otherwise

indicated, the basic structure of capacitive transducer 10 was an 80 electrode (40 each of electrodes 12 and 14) capacitor configured and dimensioned as described supra. The electrodes were of tungsten on a saphire support and were overcoated with a silicon nitride passivation layer. The capacitance of each of the resulting transducers, before further treatment, was on the order of 10 to 15 picofarads. Generally, when exposed to a dilute sample, the capacitance of each of these transducers increased to the neighborhood of 100 to 300 picofarads when observed at an intermediate radio frequency.

EXAMPLE 1.

A transducer was prepared with a reactive overcoating of potassium chromate (K_2CrO_4) covering the silicon nitride passivation layer. This was accomplished by pipetting a drop of 0.25% by weight of potassium chromate in water onto the silicon nitride surface of a horizontally held transducer and drying the transducer in a nitrogen dry box for 35 minutes.

In a series of experiments, the potassium chromate layer was exposed variously to aqueous solutions of silver nitrate (both 0.004% and 0.032%), sodium nitrate (0.025%), potassium nitrate (0.025%), and potassium chloride (0.025%), as well as to deionized water. In each test, a drop of the solution was pipetted onto the horizontal capacitor, and the change in capacitance was observed.

In all cases except tests using deionized water, increases in capacitance were observed, the capacitance rapidly reaching a peak value within about a minute following application of the fluid sample, and thereafter gradually declining to an elevated constant value over a period of several minutes. For the tests using water, a decrease in capacitance was observed, the capacitance tending to a low constant value after a period of several minutes. For both the more concentrated silver nitrate solution and the sodium nitrate, which react with potassium chromate, the capacitance change observed was greater than that observed for potassium nitrate and potassium chloride, which do not, although all of the tests showed an increase in capacitance. For the two concentrations of silver nitrate, markedly different capacitances were observed, the final capacitance for the higher concentration sample exceeding that of the lower by better than a factor of two. All of the tests were repeated a number of times, showing closely similar results.

A number of other tests were conducted using different concentrations of the reacting species. While the capacitance was found to vary in a non-linear relationship to the concentration (showing maximum sensitivity for small concentrations and tending toward a saturated condition for high), the results are repeatable, indicating that quantitation is possible through calibration measurements.

Example 1 illustrates the use of a broad-ranging reaction to permit detection of a class of species from others. The following examples are of higher specificity, relying on biochemical reactions.

EXAMPLE 2.

A pair of transducers suitable for the detection of collagenase were prepared by overcoating one with collagen (to act as the active sensor) and the other with trypsin (as a control). Each capacitor was separately coated. To effect the coating, the capacitors were heated to plus 70°C for five minutes. A drop of collagen solution containing type I collagen derived from rabbit skin in a concentration of two mg/ml in 0.5 molar acetic acid was micropipetted onto the electrode area of one capacitor. Similarly, a drop of trypsin solution consisting of 0.02% trypsin in a buffered solution of 50 mm Tris, 0.2 m NaCl, 1 mm $CaCl_2$, and 0.02% Na_3N, was placed on the other capacitor. Both transducers were then shaken to remove most of the liquid, leaving only a thin film of protein solution in place of each. The transducers were returned to an oven at plus 70°C for five minutes and then allowed to cool to room temperature in a dry nitrogen box before testing.

Capacitors so prepared were exposed variously to a buffered solution of 1 mM TRIS, 0.2 M NaCl, 1 mM $CaCl_2$, and 0.02% Na_3N at a pH of 7.4 and to a mixture of collagenase having an initial activity of 179 units per milligram mixed in the same buffer solution at a ratio of 2 microgram per milliliter. The mixtures were continuously stirred and heated to plus 37 degrees C.

A collagen- and a trypsin-coated capacitor pair were interconnected as hereinbefore indicated. On exposure to the buffer solution alone, the capacitive difference between the two sensors, as evidenced by a difference in voltage, as observed at voltmeter 44, remained constant. However, when the two capacitors were placed into the collaganese solution, an immediate change in capacitance of the collagen coated capacitor was observed. The change was monotonic, commencing rapidly (e.g., at about 100 pf/min) and then decelerating over a five-minute period to a constant elevated value of c. 250 pf.

EXAMPLE 3.

A pair of transducers designed to detect bovine serum albumin (BSA) through a pepsin-BSA reaction were prepared by micropipetting, at room temperature, pepsin and collagenase (as a control) respectively onto a pair of capacitors, shaking off the excess fluid, and allowing the thine proteinaceous coating to dry at room temperature for one hour in a dry nitrogen box. The collagenase was prepared as in Example 2. The pepsin was diluted at a rate of 50 micrograms per millileter in 0.5 molar acetic acid. For purposes of testing, the collagenase coated capacitor was dimensioned to have approximately half the capacitance of the pepsin coated capacitor, its ambient temperature capacitence being measured at 6 picofarads.

Test solutions of bovine serum albumin, two

milligrams BSA per milliliter in 0.5 molar acetic acid and collagen (type 1 rabbit skin), 2 milligrams per milliliter in 0.5 molar acetic acid were made up in addition to the trypsin and collagenase test solutions of Example 2.

The pepsin-coated transducer was exposed variously to the collagen and BSA solutions, showing substantially no reaction with the former, but a steadily varying capacitance, as evidenced by a linear changing voltage across a bridge circuit, on exposure to the latter.

The collagenase coated sensor was exposed variously to trypsin, collagen, and collagenase, showing no change on exposure to trypsin and changes of opposite sense and approximately equal (e.g., c. 25 pf/min) rates on exposure to collagen and collagenase.

Examples 2 and 3 demonstate that a transducer sensitive to concentrations of a substrate or an enzyme may be realized by respectively coating the transducer with an enzyme or its substrate.

It might be noted that the use of a substrate, rather than an enzyme, as the reactive overcoating is preferred, since such an embodiment more readily allows a high (and therefore, not reaction-limiting) substrate concentration, insuring a "zero-order" reaction. However, as indicated, either structure is possible.

EXAMPLE 4.

A pair of transducers were respectively provided with reactive coatings of human albumin and human antihemophilic factor (factor 8), the latter transducer being intended as a control. The coatings were applied from solutions of albumin and factor 8 which were each prepared in equal weight-to-volume concentrations of 0.02 grams per milliliter of 0.9% NaCl. Each transducer was prepared by applying a drop of solution and then shaking to remove excess solution, leaving a thin film. Each transducer was then heated under a lamp to evaporate the remaining liquid. After five or ten minutes, the evaporation was observed to be complete, and a characteristically white film of protein could be seen on each transducer.

The transducers were immersed in 10 milliliters of 0.9% NaCl IV solution. The solution was kept at approximately 70°F (20°C) and stirred continuously during the test. For measurements, each transducer was connected to a capacitance bridge, and the resulting two signals were differenced before being recorded. The circuit was balanced to approximately equilibrate the signals from each capacitor's bridge circuit.

For the first ten to fifteen minutes, a steady and approximately equal drift was observed on both channels, presumably due to a removal into solution of some of the protein coating (as also evidenced by the appearance of the transducer). At the end of fifteen minutes, the circuits were readjusted to zero. After this equilabration, 0.1 cc of 10% goat human albumin antiserum was micropipetted into the ten milliliters of NaCl solution, to produce an effective concentration of 0.1% antiserum. Within a minute after adding the antiserum, the drift of the albumin-coated transducer reversed its slope, while the factor 8 coated transducer continued to drift without change.

EXAMPLE 5.

Transducers were prepared using rabbit antiserum to human chorionic gonadotropin (HCG antiserum) in distilled water, obtained as the reagent of the home pregnancy test kit distributed to Ortho Pharmaceutical Corporation of Raritan, New Jersey, under the trade mark "Daisy 2". A drop of the reagent was micropipetted onto the sensor surface, excess was removed by shaking, and allowed to dry in a dry nitrogen box.

The resulting transducer was connected into a capacitance bridge circuit, and placed in a horizontal position with the dried HCG antiserum uppermost.

For an HCG-containing sample, a first morning urine sample was obtained from a human female approximately three months pregnant. A control sample was obtained from a human male.

The transducer was tested by successively exposing it to the control and HCG-containing sample by micropipetting a drop of one or the other, washing the sensor in deionized water and drying in a nitrogen atmosphere between tests. In all cases, the capacitance increased; however, for the HCG-containing sample, approximately a two minute induction time was observed.

Examples 4 and 5 are offered by way of example that transducers made in accordance with the present invention may incorporate either an antibody or antigenic coating to detect an antigen-antibody reaction.

EXAMPLE 6.

Transducers were provided with a reactive overcoating of soybean-casein digest agar, USP, secured from GIBCO diagnostics of Madison, Wisconsin as Tryptic Soy Agar. The agar gel was cut with a steril knife into sheets the dimensions of a single transducer and having a thickness of approximately 0.005 inch (0.125 mm).

A series of tests were performed with the resulting transducers, the transducers being sealed in a closed container heated to 35°C±1°C for the tests.

Prior to one set of tests, a petri dish of the agar was contaminated with human saliva and allowed to incubate in the dark at 37°C for 18 hours. At the end of this period, about one third of the growth medium was observed to be supporting microbiological growth. Cuttings approximately one-quarter covered with visible growth were obtained from this contaminated medium and used to overcoat a set of transducers. These were electrically compared with transducers covered with uncontaminated agar. While the capacitance of both contaminated and uncontaminated transducers was observed to change with time (reflecting perhaps effects of humidity or

microbiological growth even on the not deliberately contaminated transducer), the contaminated transducers were observed to have a greater rate of change, by a factor of at least two.

In another set of tests, sterile soybean-casein digest agar was used to overcoat a pair of transducers, one of which was then contaminated with human saliva. The transducers were sealed in a closed container heated to 37°C±1°C. About 30 minutes after the saliva was applied to one of the sensors, the rates of change of capacitance of both transducers were observed to be substantially equal. After 40 minutes, the rate of change of capacitance of the contaminated capacitor was observed to accelerate to about twice the rate of the uncontaminated, the acceleration itself increasing with time.

Example 6 is offered as an example of the detection of microbiological growth through the interaction of organisms and a culture medium.

It will be understood that various modifications can be made to the apparatus and methods of the present invention. Thus, while reactive overcoating 26 may be merely coated onto transducer 10 and allowed to dry, as in the examples, it will be understood that reactive overcoating 26 may be attached to the transducer by any of a number of other means. For example, reactive overcoating 26 may be chemically bound to transducer 10. Thus, if reactive overcoating 26 is antigen or antibody, it may be covalently bound to dielectric layer 24 in such a way as not to hinder the coating's reactivity in forming the antibody-antigen complex. As an example, an antibody such as immunoglobulin G (IgG) may be bound by its carboxyl terminations to silicon-containing materials by a silyl compound such as 3-aminopropyltrimethoxysilane, thereby leaving the antibody's antigen reactive amino terminations free. The method for preparing the surface of dielectric layer 24, of attaching the silyl compound thereto, and of covalently bonding an antibody to the glass through the silyl coupling, are described by Weetall (U.S. Patent 3,652,761), where may also be found a description of other silyl compounds and the methods by which carboxyl, amino, and other reactive groups of proteins, including antibody or antigen (or their fragments), may be covalently bound to various inorganic materials. An extensive art for immobilizing antigens or antibodies (and other proteins) to polymers also exists, and those skilled in the art will understand that coupling sites for antigen or antibody might be provided on polymeric dielectric layers also. Thus, for instance, if dielectric layer 24 is of nylon (polyamide), the coupling may be in the form of the substitution of an appropriate radical for the hydrogen bound to either the carbon or nitrogen of the molecular chain.

It might also be noted that the coupling material binding reactive overcoating 26 to transducer 10 may also incorporate spacer groups, as are well known in the art, to insure sufficient separation between the dielectric layer 24 and the reactive portion of the reactive material so as to minimize steric hindrance of the antibody-antigen binding process. For example, the coupling material might include a polyethylene chain, as for example in the case of 1,6 diaminohexane or 6 aminohexanoic acid bound to dielectric layer 24 through a peptide bond and respectively providing a free primary amino and a free carboxyl group for covalently binding to the carboxyl or amino termination of a protein moiety. Either of these coupling materials provide a 6-carbon chain between terminations, thereby further spacing the reactive sites of reactive overcoating 26 from dielectric layer 24 by the corresponding distance. Similar appropriate coupling and spacer materials are well known in the arts of both immunoassay and affinity chromatography.

It will be understood that other modifications can be made to the apparatus and methods of the present invention. Thus, a multiplicity of transducers with differing reactive overcoatings may be used to perform multiple or panel-type assays to quantitate a number of species or identify an unknown material suspected to be one of several possible materials. The simplest type of such an apparatus is illustrated in Figure 4, where it may be seen that transducer 110 incorporates a pair of interdigitated capacitors 112 and 114, sharing a common support 116. Capacitors 112 and 114 may in all respects be similar in structure to the capacitor described hereinbefore with regard to transducer 10, with the exception that capacitors 112 and 114 are respectively provided with reactive overcoatings 118 and 120. Reactive overcoatings 118 and 120 are chosen to be differently reactive. For example, reactive overcoatings 118 and 120 may be different growth media, possibly containing different inhibitors, as is well known in the art, thereby extending the apparatus of Example 6 to the detection and quantitation of specific bacteria in a mixed flora. Thus, reactive overcoatings 118 and 120 may be similar in composition to one another with the exception that one additionally incorporates an inhibitor. For instance, crystal violet or penicillin may be incorporated into reactive overcoating 118, thereby inhibiting the growth of Gram-positive bacteria without affecting Gram-negative species. Similarly, potassium tellurite may be added to a growth medium to inhibit the growth of Gram-negative species. Yet other selective media, variously containing either inhibitors or necessary nutrients, as are well known in the microbiological arts, may be used. Similarly, various enzyme substrate might be used to perform comparative assays, and various chemically reactive overcoatings, to perform systematic qualitative assays. The number of individual capacitors and differing reactive coatings sharing a common support clearly may be varied to suit the contemplated assay. It will also be understood that transducer 110 may be configured such that one of the

plurality of capacitors is contained in a separate compartment with a reference fluid of pre-established concentration, as taught, for example, in copending U.S. Application Serial Number 399,126, filed July 15, 1982.

Still another alternative capacitor structure is depicted in Figure 5. Transducer 210 is similar in general structure to transducer 10, with the notable exception of insulating dielectric coating 224, which surrounds each electrode 12 and 14 (and buss 16 and 18), rather than merely overlying the electrodes and extending between them, as does coating 24. Dielectric coating 224 may be of similar composition as coating 24. Reacting layer 226, of composition similar to layer 26, is applied so as to penetrate to support 222 between electrodes 12 and 14. Such a structure may be made by sequentially depositing (and etching, if required) layers of dielectric material, conductive material, and reactive material on support 222, as will be understood by those skilled in the art of printed circuit and microcircuit fabrication. This alternative construction provides for greater changes in capacitance as a result of reactions between layer 226 and the sample than does the structure of transducer 10. It will also be appreciated that this structure with foraminous support 222 and reactive layer 226 permits the circulation of sample through transducer 210.

Then again, for certain applications, the provision for simultaneously electrically isolating the electrodes of transducer 10 from the sample and providing a large value of the series capacitance due to the dielectric layer 24 and the reactive overcoating 26 may best be met by a modification of the structure to include a plurality of dielectric overcoatings, one chosen for its dielectric constant, the other for its insulating properties. As an example of a multilayered dielectric overcoating, an inner layer may be, as disclosed hereinbefore, a layer of silicon nitride (room temperature dielectric constant ~8.6, resistivity $\sim 10^{16}$ ohm-cm), while this in turn may be over coated with polyimide (room temperature dielectric constant ~3.4, resistivity $\sim 10^{17}$ ohm-cm). This latter layer is in turn overcoated with reactive overcoating 26. By the use of such multiple layers, it will be understood that a wide variety of series resistance and capacitance may be achieved.

It will also be apparent to those skilled in the art that various other modifications to the present invention may be made without departing from the scope of the disclosure. Thus, as noted hereinbefore, the capacitances of the measuring and reference capacitors may be selected to be different from one another. Again, dielectric layer 31 may be omitted.

Further, it should be noted that the geometries of the capacitors may be different than shown herein. A comb-like interdigitated electrode structure is not required, and might be replaced by, for instance, a plurality of concentrically arranged electrodes. Nor need the capacitors be fabricated by micro circuit techniques; the electrodes and busses might instead be printed, as for instance by silk screening, on a substrate. It will also be understood, particularly in this last noted variation, that the substrate might be a polymeric material.

It will also be understood that the examples given are by way of explanation, and not limitation, and that the system is adaptable to a variety of fluids, including liquids and gasses, and to the measurement of concentrations of various species of inorganic, organic, and biological materials in solutions and suspensions, to the detection of contaminants or pollutants, and to the monitoring of chemical processes, both in discrete samples and in flow streams.

Since these and other changes may be made in the above apparatus without departing from the scope of the invention herein involved, it is intended that all matter contained in the above description or shown in the accompanying drawing shall be interpreted in an illustrative and not a limiting sense.

CLAIMS

1. Apparatus for performing a selected assay from the group of assays comprising chemical assays, bioassays, and the like, wherein a chemical or biological species in a fluid is detected or quantitated or wherein a chemical or biochemical reaction in a fluid is observed, said apparatus comprising, in combination:
   - a supporting structure of a material substantially electrically nonconductive;
   - a plurality of juxtaposed electrically conductive members disposed on said supporting structure;
   - at least one dielectric disposed so as to electrically insulate said conductive members from said fluid, said dielectric being of a substance substantially chemically inert with regard to said fluid; and
   - a reactive layer disposed adjacent said conductive members and separated therefrom by said dielectric, said reactive layer being chosen to enter into a reaction with the species to be assayed.

2. Apparatus as claimed in claim 1 wherein said reactive layer comprises a substrate to an enzyme.

3. Apparatus as claimed in claim 1 wherein said reactive layer comprises an antibody.

4. Apparatus as claimed in claim 1 wherein said reactive layer comprises a reactive antibody fragment.

5. Apparatus as claimed in claim 1 wherein said reactive layer comprises an antigen.

6. Apparatus as claimed in claim 1 wherein said reactive layer comprises a hapten.

7. Apparatus as claimed in claim 1 wherein said reactive layer comprises a microbiological growth medium.

8. Apparatus for performing a panel of assays selected from the group of assays comprising chemical assays, bioassays, and the like, wherein

the relative concentrations of a plurality of chemical or biological species in a fluid are determined through their varying reactivities, said apparatus comprising, in combination:

a supporting structure of a material substantially electrically nonconductive;

a plurality of capacitors, each formed of a plurality of juxtaposed electrically conductive members disposed on said supporting structure;

at least one dielectric disposed so as to electrically insulate said conductive members from said fluid, said dielectric being of a substance substantially chemically inert with regard to said fluid; and

a plurality of reactive layers, each disposed to confront said conductive members of a respective capacitor and separated therefrom by said dielectric, said reactive layers being chosen to enter into selected and varied reactions with the species to be assayed.

9. Apparatus as claimed in claim 8 wherein said reactive layers comprise substrates to a plurality of enzymes.

10. Apparatus as claimed in claim 8 wherein said reactive layers comprise a pluralty of antibodies.

11. Apparatus as claimed in claim 8 wherein said reactive layers comprise a plurality of reactive antibody fragments.

12. Apparatus as claimed in claim 8 wherein said reactive layers comprise a plurality of antigens.

13. Apparatus as claimed in claim 8 wherein said reactive layers comprise a plurality of haptens.

14. Apparatus as claimed in claim 8 wherein said reactive layers comprise a plurality of microbiological growth media.

15. Method for performing a selected assay from the group of assays comprising chemical assays, bioassays, and the like, wherein a chemical or biological species in a fluid is detected or quantitated or wherein a chemical or biochemical reaction in a fluid is observed, said method comprising, in sequence, the steps of:

providing at least a part of a capacitor with a dielectric comprising at least in part a reactive dielectric, said reactive dielectric being chosen to enter into a reaction with the species to be assayed;

exposing said reactive dielectric to a sample to be assayed; and

observing the capacitance change of said capacitor.

16. Apparatus for performing a selected assay or a panel of assays substantially as herein described with reference to and as illustrated in the accompanying drawings.

17. A method for performing a selected assay substantially as herein described and illustrated.

Printed in the United Kingdom for Her Majesty's Stationery Office, Demand No. 8818935, 9/1984. Contractor's Code No. 6378.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.